

ISOLATION OF CAFFEINE FROM TEA BAGS***Jindal Diksha, Singh Jarnail, Patil R.K. and Patil H.C.**

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ABSTRACT

Caffeine extracted and characterized from tea bags. Isolation was done by liquid-liquid extraction method. The extraction was done in four steps: steeping, evaporation, liquid-liquid extraction and distillation. Here the organic solvent dichloromethane is used to extract caffeine from an aqueous extract of tea leaves because caffeine is more soluble in dichloromethane (140 mg/ml) than it is in water (22 mg/ml). Calcium hydroxide is added to the extraction medium to ensure that the acidic components in the tea bags remain water soluble and that caffeine is the free base. However, the tannins that are slightly soluble in dichloromethane can be eliminated by converting it to their salts (phenolic anions by adding sodium carbonate) (tannins are phenolic compounds of high molecular weight and being acidic in nature can be converted to salts by deprotonation of the -OH group) which remain in the water. The technique used for purity analysis was done using melting point analysis. The melting point of caffeine extracted from tea bags was found to be 238°C. The purity showed that the results that the extracted caffeine was 90% pure.

KEYWORDS: Caffeine, Xanthine alkaloids, Dichloromethane, tea bags, Melting point.**INTRODUCTION**

Shen-Nung also known as the "Divine Healer" is a Chinese emperor who discovered Tea. The legend says that in the Emperor's pot of boiling water some tea leaves had fallen off. Today, much of the world's teas are growing thousands of feet above sea level. The leading tea - producing countries are Argentina, China, India, Indonesia, Japan, Kenya, Malawi, Sri Lanka, Tanzania and Taiwan (Tea Fact Sheet, 2015).

One of tea's main ingredients is caffeine. Caffeine makes you more alert by energizing your central nervous system. Tea is naturally low in caffeine, for example, the black tea has only 40 mg of caffeine but this dosage is enough for stimulating your body.

Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of more than 63 plants species worldwide. The most common sources of caffeine are coffee (*Coffea* spp.), cocoa (*Theobroma cacao*) beans, cola nuts (*Colavera*), tea (*Camellia sinensis*) leaves, guarana (*Paullinia cupana*) berries, yerba mate, yaupon holly. The amount of caffeine found in these products varies - the highest amounts are found in guarana (4-7%), followed by tea leaves (3.5%), mate tea leaves (0.89 -1.73%). Caffeine is the most widely consumed psychoactive substance and can be a mild central nervous system stimulant. It does not accumulate in the body over a period of time and is normally excreted

within several hours of consumption (Barone and Roberts, 1996). Caffeine and other purine alkaloids, including theobromine and theophylline, have played a major role in the long standing popularity of non alcoholic beverages and foods such as coffee, tea, cocoa, mate, chocolate and a wide range of soft drinks (Asahihara 2008).

Caffeine is an alkaloid of the methylxanthine family, thus it is known as 1, 3, 7- trimethylxanthine. Caffeine is an intensely bitter white powder in its pure state. Its IUPAC name is 1, 3, 7- trimethyl -1 H- purine - 2, 6 (3H, 7H) - dione, with chemical formula $C_8H_{10}N_4O_2$ (Arnaud, 1987).

Caffeine is absorbed orally with a max blood peak after 120 mins spreading quickly in all tissues. Caffeine is classified as a stimulant because it increases the activity of cardiovascular, digestive and sympathetic nervous system, and produces the sense of alertness in the brain. it can have a lethal effect (acute intoxication) when ingested at amounts of 1-5g, with plasma concentrations higher than 80mg/ml and the first intoxication signs appear at about 250 mg.

MATERIALS AND METHOD**Collection of samples**

Determination of caffeine in tea bags is the major objectives of this study. Tea bags used in this study were

obtained from a supermarket of Bathinda. All the solvents and reagents were of analytical grade.

Extraction of caffeine from tea bags

To determine the amount of caffeine in tea bags, different techniques were performed. Firstly, placed a 500ml beaker and added 2 grams of calcium hydroxide were added to ensure that caffeine remains present as the free base. The solution was brought to a boil and one bag of Lipton tea was added. 10 ml of distilled water was added and the bag was left so dipped for 5 minutes. After the 5 minutes, the tea bag was squeezed to remove excess water and second bag was added in its place. The second bag was left to dip for 5 minutes and then removed from the beaker after being carefully squeezed to remove excess water. This liquid-liquid extraction method was performed to separate caffeine into the solvent. The extract was then poured into a separating funnel, to ensure proper mixing and placed in an ice bath to cool. 3 portions of 2 ml dichloromethane were then used to extract the caffeine from the solution. The separating funnel was shaken carefully after each portion was added in order to mix the solvents while avoiding emulsions. One portion was added, and pipette was used

to remove the bottom layer that contained dichloromethane and caffeine and place it in a 50 ml beaker. This process was repeated to more times for the two remaining portions of dichloromethane. There were only a few brown specks in the solution, so a quick swirl motion of the beaker cause them to stick to the sides. The dichloromethane organic layer that contained the caffeine was transferred to another 50ml beaker and a drying agent in the form of anhydrous sodium sulphate beads was added in order to remove excess water. After that, it was decant to a 250 ml round bottom flask. Boiling chips were then added for the distillation process. The extraction solvent was then distilled from the caffeine using hot plate as the heat source. The temperature was monitored to prevent the overheating of the caffeine and making the extraction solvent as the only distilled substance. The heat source was then removed to halt the distillation process. The flask found in the other end is the dichloromethane; its volume was measured and was discarded after. Using an acetone the ball flask was rinsed. Then it was poured into the watch glass and exposed to open air. The crude caffeine mass was then measured.

RESULTS AND DISCUSSION

Tabel-1 Extraction (Solid-Liquid and Liquid-Liquid).

| | |
|--------------------|----------|
| Mass of Tea Powder | 10.1540g |
| Sodium chloride | 20.6193g |
| Calcium Hydroxide | 1.0329g |

Tabel-2 Distillation.

| | |
|--|----------|
| Mass of Watch Glass | 36.8642g |
| Mass of Watch Glass with Caffeine Crystals | 36.8989g |
| Mass of Caffeine | 0.0347g |
| % Recovery | 0.3417% |

$$\begin{aligned} \text{Percentage recovery} &= (\text{Mass of caffeine}/\text{Mass of tea}) \times 100\% \\ &= 0.0347 \text{ g} / 10.1540 \text{ g} \times 100\% \\ &= 0.3417 \% \end{aligned}$$

The structure of the caffeine extracted from the tea leaves deeply impacts the functions it performs (Wang, 2011). Essentially, caffeine is a purine with three functional groups: an amine, amide, and an alkene. The basic property of caffeine comes from the lone pair of electrons found around the nitrogen(s) (NCBI, 2013). It is an achiral molecule and does not have any stereoisomers. Caffeine is also a polar molecule; this is evident because of the London dispersion forces, dipole-dipole interactions, and hydrogen bonding present when it is in water. It also has a very hydrophobic region (NCBI, 2013). The nitrogen present in caffeine controls solubility. Caffeine is soluble in water at approximately 2.2 mg/ml at 25°C, 180 mg/ml at 80°C, and 670 mg/ml at 100°C (Williamson, 2011). It is an organic molecule that has the properties of an organic amine base (Tello, 2011). When extracting caffeine; the water was kept at a high temperature in order to increase solubility of caffeine in water to about 670 mg/ml at 100°C. Boiling

chips were added to the solution in order to prevent "bumping" and enable the smooth formation of bubbles when boiling occurs. The solution was later cooled to a lower temperature in order to impact the solubility once more and to minimize the attraction to the aqueous layer while in the separatory funnel (Williamson, 2011). The solution was also cooled before the dichloromethane was added because dichloromethane has a boiling point of 40°C. If the cold water was not added to lower the temperature, the dichloromethane would have evaporated and caffeine would not be properly extracted (Dullo, 2008). During the solid/liquid extraction the solid insoluble material such as cellulose is separated from caffeine and tannins, which are water soluble. In order to isolate caffeine a difference in solubility must occur to separate the tannins into the aqueous layer. Sodium carbonate is added to the extraction medium to ensure that the acidic components in the tea leaves remain water soluble and that caffeine is the free base. Sodium

carbonate is basic. Tannins are acidic compounds with a high molecular weight that have an –OH directly bound to an aromatic ring. Because tannins are acidic and can be converted to phenolic salts by deprotonation of the –OH group when a base is added, it is possible to separate the tannins from caffeine (Dullo, 2008). Sodium carbonate serves two main functions: to place caffeine in a more basic environment so that it has a higher affinity for dichloromethane and to cause the tannins to form phenolic salts in the aqueous solution. Adding something basic to caffeine will make it more neutral, and the “like dissolves like” idea can be applied. In this situation, the sodium carbonate acts as a nucleophile and the tannin is an electrophile. Nucleophile attacks electrophile. It is basically an acid/base reaction. The aqueous layer (density of 1 g/ml) contained dissolved tannin salts and chlorophyll. Dipole dipole interactions, London dispersion forces, hydrogen bonding, and ionic bonding with the salts took place.

When dichloromethane was added to extract caffeine from the aqueous solution, two immiscible layers formed: an organic and aqueous layer. In this instance, caffeine is usually a polar substance, but it becomes significantly less polar when it is in a basic solution. Therefore, it is soluble in dichloromethane and suspends in the organic layer. Dichloromethane is an alkyl halide and is denser than water, so it is located at the bottom of the separatory funnel. It has a density of 1.325 g/ml. It had chloro functional groups that make it susceptible to both substitution and elimination reactions (Oneota, 2003). The concentration of the solutes in the organic layer also contributes to the fact that it is located below the aqueous layer. There is a high concentration of caffeine, reactants (because the reaction does not go to 100% completion), and small amounts of water. The intermolecular forces in the organic layer are van der Waals interactions, dipole dipole moments, and London forces. Caffeine was extracted with dichloromethane in order to “wash” it three separate times to obtain as much of the pure sample. Emulsions are small droplets of the organic layer that are suspended in the aqueous that are a result of vigorous shaking of the separatory funnel (Williamson, 2011). There are numerous ways to remove emulsions, though the best form is prevention. However, they can be broken after a sufficient amount of time. The aqueous layer can also be made more ionic, and centrifugation works very well especially on a microscale level. A drying agent was added to the organic layer because dichloromethane dissolved not only the caffeine, but water as well. The drying agent, anhydrous CaCl₂ was added to remove excess water so that a pure sample of caffeine could be obtained after the solvent evaporated at room temperature (Williamson, 2011). Anhydrous calcium chloride has a high affinity for water, and then reverses back to the hydrated form after it has absorbed the water. Calcium chloride is a preferred drying agent because the pellets form clumps when excess water is present that make it simple to identify how much drying agent is

needed (Williamson, 2011). The pellets stopped clumping together when excess water was removed. It is also very rapid, effective, and ideal for microscale experiments. In order to remove the dichloromethane, the beaker could be placed in a hot water bath so that the solvent would evaporate and leave a pure sample. Sublimation is a technique that may have been used to produce a purer caffeine sample, but it could have led to a higher loss of product. Liquid-liquid extractions were used to transfer a solute from one solvent to another and isolate desired product.

The total amount of caffeine extracted was 0.0347 g and the calculated percent recovery was 0.3417 %. This was the amount of caffeine extracted from the crude caffeine in the tea bags. This demonstrates that there was a significant amount of product lost throughout the procedure. It is also important to consider that the reaction cannot go to completion, so 100% yield is not possible. A loss of product could have occurred due to emulsions and due to not thoroughly “washing” with dichloromethane to extract as much caffeine as possible. There was a lot of transfer throughout the procedure, which presented many opportunities to lose product. It is also possible that the concentration of caffeine was not high enough because too much water was added. A systematic error with the scales was observed due to a lack of calibration, this could have affected the measurement of the final product. Another source of error could be the theoretical amount of caffeine in the tea bags, if it was more or less due to random error, the percent recovery would be calculated differently. The overall percent error was about 40.9 %. This number could be skewed due to measurement errors of the crude product. In order to reduce sources of error in the future, two trials could be done. It is also possible to use a different source of caffeine, and to ensure all techniques are performed properly.

CONCLUSION

Caffeine was the final product. Overall, a total of 0.0347 g was obtained from a possible amount of 10.1540g tea. The total percent recovery was 0.3417 %. This number reflects on how erroneous the procedure was performed. Many sources of error may have occurred such as an incorrect calibration of the scales with which the samples were measured and not “washing” the solution thoroughly enough to obtain as much sample as possible. It was not possible to obtain 100% recovery because the reaction never goes 100 % to completion and because of material loss through transfer during the procedure.

Use the Lipton brand of tea because it has no coloring and you can really see the clear layer.

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